



**EXHIBIT A**

**PENDING CLAIMS UPON ENTRY OF THE  
INSTANT AMENDMENT  
(filed August 24, 2000)**

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SEP 03 2002

TECH CENTER 1600/2900

**U.S. PATENT APPLICATION SERIAL NO. 09/645,415**

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2. An attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more primary effector molecules and one or more secondary effector molecules operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe.
3. The attenuated tumor-targeted bacteria of claim 1 or 2, wherein at least one of the primary effector molecules is a TNF family member.
4. The attenuated tumor-targeted bacteria of claim 3, wherein the TNF family member is tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), TNF- $\alpha$ -related apoptosis-inducing ligand (TRAIL), TNF- $\alpha$ -related activation-induced cytokine (TRANCE), TNF- $\alpha$ -related weak inducer of apoptosis (TWEAK), CD40 ligand (CD40L), LT- $\alpha$ , LT- $\beta$ , OX40L, CD40L, FasL, CD27L, CD30L, 4-1BBL, APRIL, LIGHT, TL1, TNFSF16, TNFSF17, or AITR-L.
5. The attenuated tumor-targeted bacteria of claim 1 or 2, wherein at least one of the primary effector molecules is an anti-angiogenic factor.
6. The attenuated tumor-targeted bacteria of claim 5, wherein the anti-angiogenic factor is endostatin, angiostatin, anti-angiogenic antithrombin III, the 29 kDa N-terminal and a 40 kDa C-terminal proteolytic fragments of fibronectin, a uPA receptor antagonist, the 16 kDa proteolytic fragment of prolactin, the 7.8 kDa proteolytic fragment of platelet factor-4, the anti-angiogenic 24 amino acid fragment of platelet factor-4, the anti-angiogenic factor designated 13.40, the anti-angiogenic 22 amino acid peptide fragment of thrombospondin I,

the anti-angiogenic 20 amino acid peptide fragment of SPARC, RGD and NGR containing peptides, the small anti-angiogenic peptides of laminin, fibronectin, procollagen and EGF, and peptide antagonists of integrin  $\alpha_v\beta_3$ , or VEGF receptor.

7. The attenuated tumor-targeted bacteria of claim 1 or 2, wherein at least one of the primary effector molecules is a bacteriocin family member with the proviso said bacteriocin is not BRP.

8. The attenuated tumor-targeted bacteria of claim 7, wherein the bacteriocin family member is ColE1, ColE1a, ColE1b, ColE2, ColE3, ColE4, ColE5, ColE6, ColE7, ColE8, ColE9, Colicin A, Colicin K, Colicin L, Colicin M, cloacin DF13, pesticin A1122, staphylococcin 1580, butyricin 7423, pyocin R1 or AP41, megacin A-216, vibriocin, or microcin M15.

9. The attenuated tumor targeted bacteria of claim 1 or 2, wherein the primary effector molecule is a tumor inhibitory enzyme.

10. The attenuated tumor targeted bacteria of claim 9, wherein the tumor inhibitory enzyme is methionase, asparaginase, lipase, phospholipase, protease, DNAase or glycosidase.

11. The attenuated tumor targeted bacteria of claim 1 or 2, wherein the primary effector molecule is hemolysin, verotoxin, CNF1, CNF2, or PMT.

12. The attenuated tumor-targeted bacteria of claim 1 or 2, wherein the primary effector molecule is derived from an animal, plant, bacteria, or virus.

13. The attenuated tumor-targeted bacteria of claim 2, wherein the secondary effector molecule is an immunomodulating agent, an anti-tumor protein, a pro-drug converting enzyme, an antisense molecule, a ribozyme, or an antigen.

14. The attenuated tumor-targeted bacteria of claim 1 or 2, wherein the attenuated tumor-targeted bacteria is *Salmonella*.

16. The attenuated tumor-targeted bacteria of claim 2, wherein the secondary effector molecule is a bacteriocin release factor (BRP).

26. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more primary effector molecules and one or more secondary effector molecules operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe.

27. The pharmaceutical composition of claim 25 or 26, wherein at least one of the primary effector molecules is a TNF family member.

28. The pharmaceutical composition of claim 27, wherein the TNF family member is tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), TNF- $\alpha$ -related apoptosis-inducing ligand (TRAIL), TNF- $\alpha$ -related activation-induced cytokine (TRANCE), TNF- $\alpha$ -related weak inducer of apoptosis (TWEAK), CD40 ligand (CD40L), LT- $\alpha$ , LT- $\beta$ , OX40L, CD40L, FasL, CD27L, CD30L, 4-1BBL, APRIL, LIGHT, TL1, TNFSF16, TNFSF17, or AITR-L.

29. The pharmaceutical composition of claim 25 or 26, wherein at least one of the primary effector molecules is an anti-angiogenic factor.

30. The pharmaceutical composition of claim 29, wherein the anti-angiogenic factor is endostatin, angiostatin, anti-angiogenic antithrombin III, the 29 kDa N-terminal and a 40 kDa C-terminal proteolytic fragments of fibronectin, a uPA receptor antagonist, the 16 kDa proteolytic fragment of prolactin, the 7.8 kDa proteolytic fragment of platelet factor-4, the anti-angiogenic 24 amino acid fragment of platelet factor-4, the anti-angiogenic factor designated 13.40, the anti-angiogenic 22 amino acid peptide fragment of Thrombospondin I, the anti-angiogenic 20 amino acid peptide fragment of SPARC, RGD and NGR containing peptides, the small anti-angiogenic peptides of laminin, fibronectin, procollagen and EGF, and peptide antagonists of integrin  $\alpha_v\beta_3$ , or VEGF receptor.

31. The pharmaceutical composition of claim 25 or 26, wherein at least one of the primary effector molecules is a bacteriocin family member with the proviso said bacteriocin is not BRP.

32. The pharmaceutical composition of claim 31, wherein the bacteriocin family member is ColE1, ColE1a, ColE1b ColE2, ColE3, ColE4, ColE5, ColE6, ColE7, ColE8, ColE9, Colicin A, Colicin K, Colicin L, Colicin M, cloacin DF13, pesticin A1122, staphylococcin 1580, butyricin 7423, pyocin R1 or AP41, megacin A-216, vibriocin or microcin M15.

33. The pharmaceutical composition of claim 25 or 26, wherein at least one of the primary effector molecule is a tumor inhibitory enzyme.

34. The pharmaceutical composition of claim 33, wherein the tumor inhibitory enzyme is methionase, asparaginase, lipase, phospholipase, protease, DNAase or glycosidase.

35. The pharmaceutical composition of claim 25 or 26, wherein at least one of the primary effector molecule is hemolysin, verotoxin, CNF1, CNF2, or PMT.

36. The pharmaceutical composition of claim 25 or 26, wherein the primary effector molecule is derived from an animal, plant, bacteria, or virus.

37. The pharmaceutical composition of claim 26, wherein the secondary effector molecule is an immunomodulating agent, an anti-tumor protein, a pro-drug converting enzyme, an antisense molecule, a ribozyme, or an antigen.

38. The pharmaceutical composition of claim 25 or 26, wherein the attenuated tumor-targeted bacteria is *Salmonella*.

40. The pharmaceutical composition of claim 26, wherein the secondary effector molecule is a bacteriocin release factor.

49. A method for delivering one or more primary effector molecules and one or more secondary effector molecules for the treatment of a solid tumor cancer to a subject in need of such treatment, comprising administering a pharmaceutical composition a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more primary effector molecules and one or more secondary effector molecules operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe.

50. The method of claim 48 or 49, wherein at least one of the primary effector molecules is a TNF family member.

51. The method of claim 50, wherein the TNF family member is tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), TNF- $\alpha$ -related apoptosis-inducing ligand (TRAIL), TNF- $\alpha$ -related activation-induced cytokine (TRANCE), TNF- $\alpha$ -related weak inducer of apoptosis (TWEAK), CD40 ligand (CD40L), LT- $\alpha$ , LT- $\beta$ , OX40L, CD40L, FasL, CD27L, CD30L, 4-1BBL, APRIL, LIGHT, TL1, TNFSF16, TNFSF17, or AITR-L.

52. The method of claim 48 or 49, wherein at least one of the primary effector molecules is an anti-angiogenic factor.

53. The method of claim 52, wherein the anti-angiogenic factor is endostatin, angiostatin, anti-angiogenic antithrombin III, the 29 kDa N-terminal and a 40 kDa C-terminal proteolytic fragments of fibronectin, a uPA receptor antagonist, the 16 kDa proteolytic fragment of prolactin, the 7.8 kDa proteolytic fragment of platelet factor-4, the anti-angiogenic 24 amino acid fragment of platelet factor-4, the anti-angiogenic factor designated 13.40, the anti-angiogenic 22 amino acid peptide fragment of thrombospondin I, the anti-angiogenic 20 amino acid peptide fragment of SPARC, RGD and NGR containing peptides, the small anti-angiogenic peptides of laminin, fibronectin, procollagen and EGF, and peptide antagonists of integrin  $\alpha_v\beta_3$ , or VEGF receptor.

54. The method of claim 48 or 49, wherein at least one of the primary effector molecules is bacteriocin family member with the proviso said bacteriocin is not BRP.

55. The method of claim 54, wherein the bacteriocin family member is ColE1, ColE1a, ColE1b ColE2, ColE3, ColE4, ColE5, ColE6, ColE7, ColE8, ColE9, Colicin A, Colicin K, Colicin L, Colicin M, cloacin DF13, pesticin A1122, staphylococcin 1580, butyricin 7423, pyocin R1 or AP41, megacin A-216, vibriocin or microcin M15.
56. The method of claim 48 or 49, wherein at least one of the primary effector molecules is a tumor inhibitory enzyme.
57. The method of claim 56, wherein the tumor inhibitory enzyme is methionase, asparaginase, lipase, phospholipase, protease, DNAase or glycosidase.
58. The method of claim 48 or 49, wherein at least one of the primary effector molecules is hemolysin, verotoxin, CNF1, CNF2 or PMT.
59. The method of claim 48 or 49, wherein at least one of the primary effector molecules are derived from an animal, plant, bacteria, or virus.
60. (Amended) The method of claim 49, wherein at least one of the secondary effector molecule is an anti-tumor protein, an immunomodulating agent, a pro-drug converting enzyme, an antisense molecule, a ribozyme, or an antigen.
61. The method of claim 48 or 49, wherein the attenuated tumor-targeted bacteria is *Salmonella*.
63. The method of claim 49, wherein the secondary effector molecule is a bacteriocin release factor.
72. A method of treating a solid tumor cancer in an animal, comprising administering one or more chemotherapeutic agents and a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria comprising one or more nucleic acid molecules encoding one or more primary effector molecules and one or

more secondary effector molecule operably linked to one or more promoters, wherein said attenuated tumor-targeted bacteria is a facultative aerobe or facultative anaerobe.

73. The method of claim 71 or 72, wherein at least one of the primary effector molecules is a TNF family member.

74. The method of claim 73, wherein the TNF family member is tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), TNF- $\alpha$ -related apoptosis-inducing ligand (TRAIL), TNF- $\alpha$ -related activation-induced cytokine (TRANCE), TNF- $\alpha$ -related weak inducer of apoptosis (TWEAK), CD40 ligand (CD40L), LT- $\alpha$ , LT- $\beta$ , OX40L, CD40L, FasL, CD27L, CD30L, 4-1BBL, APRIL, LIGHT, TL1, TNFSF16, TNFSF17, or AITR-L.

75. The method of claim 71 or 72, wherein at least one of the primary effector molecules is an anti-angiogenic factor.

76. The method of claim 75, wherein the anti-angiogenic factor is endostatin, angiostatin, anti-angiogenic antithrombin III, the 29 kDa N-terminal and a 40 kDa C-terminal proteolytic fragments of fibronectin, a uPA receptor antagonist, the 16 kDa proteolytic fragment of prolactin, the 7.8 kDa proteolytic fragment of platelet factor-4, the anti-angiogenic 24 amino acid fragment of platelet factor-4, the anti-angiogenic factor designated 13.40, the anti-angiogenic 22 amino acid peptide fragment of thrombospondin I, the anti-angiogenic 20 amino acid peptide fragment of SPARC, RGD and NGR containing peptides, the small anti-angiogenic peptides of laminin, fibronectin, procollagen and EGF, and peptide antagonists of integrin  $\alpha_v\beta_3$ , or VEGF receptor.

77. The method of claim 71 or 72, wherein at least one of the primary effector molecules is a bacteriocin family member with the proviso said bacteriocin is not BRP.

78. The method of claim 77, wherein the bacteriocin family member is ColE1, ColE1a, ColE1b ColE2, ColE3, ColE4, ColE5, ColE6, ColE7, ColE8, ColE9, Colicin A, Colicin K, Colicin L, Colicin M, cloacin DF13, pesticin A1122, staphylococcin 1580, butyricin 7423, pyocin R1 or AP41, megacin A-216, vibriocin, or microcin M15.

79. The method of claim 71 or 72, wherein at least one of the primary effector molecule is a tumor inhibitory enzyme.

80. The method of claim 79, wherein the tumor inhibitory enzyme is methionase, asparaginase, lipase, phospholipase, protease, DNAase or glycosidase.

81. The method of claim 71 or 72, wherein at least one of the primary effector molecule is hemolysin, verotoxin, CNF1, CNF2, or PMT.

82. The method of claim 71 or 72, wherein the primary effector molecule is derived from an animal, plant, bacteria, or virus.

83. The method of claim 82, wherein the secondary effector molecule is an immunomodulating agent, an anti-tumor protein, a pro-drug converting enzyme, an antisense molecule, a ribozyme, or an antigen.

84. The method of claim 71 or 72, wherein the attenuated tumor-targeted bacteria is *Salmonella*.

86. The method of claim 72, wherein the secondary effector molecule is a bacteriocin release factor.

94. A method of treating a solid tumor cancer in an animal, comprising administering one or more chemotherapeutic agents and a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an attenuated tumor-targeted bacteria.

100. The attenuated tumor targeted bacteria of claim 2, wherein the secondary effector molecule is a release factor.

101. The attenuated tumor targeted bacteria of claim 2, wherein the antisense molecule is double-stranded or single-stranded DNA, double-stranded or single-stranded RNA, or a triplex molecule.



102. The attenuated tumor targeted bacteria of claim 2, wherein the anti-tumor protein is a ribosome inactivating protein.

103. The attenuated tumor targeted bacteria of claim 102, wherein the ribosome inactivating protein is saporin, ricin, or abrin.

104. The attenuated tumor targeted bacteria of claim 2, wherein the pro-drug converting enzyme is cytochrome p450 NADPH oxidoreductase.

105. The attenuated tumor targeted bacteria of claim 16, wherein the BRP protein is obtainable from the cloacin DF13 plasmid.

106. The attenuated tumor targeted bacteria of claim 13, wherein the immunomodulating agent is IL-1, IL-2, IL-4, IL-5, IL-10, IL-15, IL-18, endothelial monocyte activating protein-2, GM-CSF, IFN- $\gamma$ , IFN- $\alpha$ , MIP-3 $\alpha$ , SLC, or MIB-3 $\beta$ .

107. The attenuated tumor targeted bacteria of claim 13, wherein the immunomodulating agent is encoded by an MHC gene.

108. The attenuated tumor targeted bacteria of claim 107, wherein the immunomodulatory agent encoded by the MHC gene is HLA-B7.

109. The attenuated tumor targeted bacteria of claim 13, wherein the immunomodulating agent is  $\alpha$ -1,3-galactosyl transferase.

110. The attenuated tumor targeted bacteria of claim 13, wherein the immunomodulating agent is a tumor-associated antigen.

111. The attenuated tumor targeted bacteria of claim 110, wherein the tumor-associated antigen is carcinoembryonic antigen (CEA).

112. The attenuated tumor targeted bacteria of claim 2, wherein the secondary effector molecule is an inhibitor of inducible nitric oxide synthase or of endothelial nitric oxide synthase.
113. The pharmaceutical composition of claim 26, wherein the secondary effector molecule is a release factor.
114. The pharmaceutical composition of claim 37, wherein the antisense molecule is double-stranded or single-stranded DNA, double-stranded or single-stranded RNA, or a triplex molecule.
115. The pharmaceutical composition of claim 37, wherein the anti-tumor protein is a ribosome inactivating protein.
116. The pharmaceutical composition of claim 115, wherein the ribosome inactivating protein is saporin, ricin, or abrin.
117. The pharmaceutical composition of claim 37, wherein the pro-drug converting enzyme is cytochrome p450 NADPH oxidoreductase.
118. The pharmaceutical composition of claim 40, wherein the BRP protein is obtainable from cloacin DF13.
119. The pharmaceutical composition of claim 37, wherein the immunomodulating agent is IL-1, IL-2, IL-4, IL-5, IL-10, IL-15, IL-18, endothelial monocyte activating protein-2, GM-CSF, IFN- $\gamma$ , IFN- $\alpha$ , MIP-3 $\alpha$ , SLC, or MIB-3 $\beta$ .
120. The pharmaceutical composition of claim 37, wherein the immunomodulating agent is encoded by an MHC gene.
121. The pharmaceutical composition of claim 120, wherein the immunomodulatory agent encoded by the MHC gene is HLA-B7.

122. The pharmaceutical composition of claim 37, wherein the immunomodulating agent is  $\alpha$ -1,3-galactosyl transferase.

123. The pharmaceutical composition of claim 37, wherein the immunomodulating agent is a tumor-associated antigen.

124. The pharmaceutical composition of claim 123, wherein the tumor-associated antigen is carcinoembryonic antigen (CEA).

125. The pharmaceutical composition of claim 26, wherein the secondary effector molecule is an inhibitor of inducible nitric oxide synthase or of endothelial nitric oxide synthase.

126. The method of claim 49, wherein at least one of the secondary effector molecule is a release factor.

127. The method of claim 60, wherein the antisense molecule is double-stranded or single-stranded DNA, double-stranded or single-stranded RNA, or a triplex molecule.

128. The method of claim 60, wherein the anti-tumor protein is a ribosome inactivating protein.

129. The method of claim 128, wherein the ribosome inactivating protein is saporin, ricin, or abrin.

130. The method of claim 60, wherein the pro-drug converting enzyme is cytochrome p450 NADPH oxidoreductase.

131. The method of claim 63, wherein the BRP protein is obtainable from cloacin DF13.

132. The method of claim 60, wherein the immunomodulating agent is IL-1, IL-2, IL-4, IL-5, IL-10, IL-15, IL-18, endothelial monocyte activating protein-2, GM-CSF, IFN- $\gamma$ , IFN- $\alpha$ , MIP-3 $\alpha$ , SLC, or MIB-3 $\beta$ .

133. The method of claim 60, wherein the immunomodulating agent is encoded by an MHC gene.

134. The method of claim 60, wherein the immunomodulatory agent encoded by the MHC gene is HLA-B7.

135. The method of claim 60, wherein the immunomodulating agent is  $\alpha$ -1,3-galactosyl transferase.

136. The method of claim 60, wherein the immunomodulating agent is a tumor-associated antigen.

137. The method of claim 136, wherein the tumor-associated antigen is carcinoembryonic antigen (CEA).

138. The method of claim 49, wherein the secondary effector molecule is an inhibitor of inducible nitric oxide synthase or of endothelial nitric oxide synthase.

139. The attenuated tumor targeted bacteria of claim 2, wherein the pro-drug converting enzyme is cytosine deaminase.

140. The pharmaceutical composition of claim 37, wherein the pro-drug converting enzyme is cytosine deaminase.

141. The method of claim 60, wherein the pro-drug converting enzyme is cytosine deaminase.